

SAFE IMMOBILIZATION BY CO₂ OF *LATRODECTUS HESPERUS* (ARACHNIDA: THERIDIIDAE)

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Abstract.—Western black widow spiders (*Latrodectus hesperus* Chamberlin & Ivie) can be handled safely following anesthesia using CO₂ gas. The spiders remain immobile for approximately one minute for each three minutes the gas is applied. For safety, the 99% confidence interval for this ratio is four minutes of gas:one minute of immobilization. These ratios appear to be independent of size for mature females with mass greater than 100 mg.

Key Words.—Arachnida, *Latrodectus*, spider, black widow, anesthesia, CO₂.

The silks of the black widow spiders of the western United States, *Latrodectus hesperus*, Chamberlin & Ivie have unique material properties that make them worthy of study (Moore & Tran 1996). Techniques for forcible silking (Work & Emerson 1982; Thiel et al. 1994) have been used to draw silk directly from spinnerets of various spiders. These methods cannot be applied to black widows without careful consideration of a system of anesthesia to prevent toxic bites. CO₂ has been used to anesthetize spiders (Baptista 1989) and insects (Turillazzi 1992). This study was undertaken to determine the range of dosages of CO₂ that provide a useful window of immobilization time in adult female *L. hesperus*. We present an estimate of minimum CO₂ dosage per time immobilized that can be used for safe handling of this species.

METHODS AND MATERIALS

Specimens Used.—All experiments were performed on *L. hesperus* captured from several locations in Southern California, primarily the Bernard Biological Field Station of the Claremont Colleges, Claremont, and residential areas around the University of California, Riverside. Each individual was kept in its own glass tank which contained a bamboo frame to facilitate web building. The spider room was kept at a temperature of 19–21° C. The spiders were fed medium-sized cricket nymphs *ad libitum*; the spiders ate them at a rate of about one cricket per week.

Safety Precautions.—In order to prevent toxic bites, several precautions were taken. While handling and transporting the specimens, leather garden gloves and long-sleeved shirts or lab coats were worn. Specimens were transported in 400 ml plastic containers with snap-on lids of the type used to store perishable foods. It is preferable to use containers with translucent lids that allow the spider's position and activity to be seen before opening the container. Specimens were moved from web to transfer container (and back to its web following each trial) by gently prodding them with a soft-bristled paintbrush.

For several minutes after transfer to the small container, the spiders spun lines of silk to connect themselves to their new substrate. This behavior pattern included circular spinning movements of the fourth pair of legs, which we used as the

baseline behavior upon which to judge whether the spider was under anesthesia or active.

Anesthesia.—To anesthetize the spiders, CO₂ was introduced via wide-bore hypodermic needle into the container inserted in one of two pinholes in the container's lid. The regulator on the CO₂ tank was set to 2 p.s.i. The other pinhole was left uncovered to allow enough outflow of air so that the container's lid would not pop open. For each trial, each spider was thus bathed in CO₂ for 15, 25, 35 or 45 min. All trials were performed between 1000 and 1400, and all spiders were tested one at a time.

At the end of each CO₂ exposure, the lid was removed from the container to expose the anesthetized spider to air. The mass of the spider was measured during the first two minutes of anesthesia. The time from exposure to air until the return of movement was then measured. The container holding the spider was given a sharp tap at the end of each 30-sec interval during the recovery period. Preliminary experiments showed that the spiders generally required a stimulus following anesthesia to become active again. The time under anesthesia was recorded as the last 30-second "tap" before the spider resumed the circular leg-movements seen before CO₂ application. The specimens resumed normal activity upon being replaced in their original webs following the trials, including web maintenance, prey capture, and egg case production.

With the exception of one specimen which died between the first and second trial, and one which was available only for the 35 and 25 minute tests, all trials were done on the same set of spiders. Experiments were performed at least one week apart, and with a minimum of one feeding between tests, to allow full recovery. Between the first and second trial, one specimen produced an egg case.

Regressions, correlations and curvefit analyses were done with SPSS for Macintosh and repeated using StatMost 32 for Windows.

RESULTS AND DISCUSSION

The relationship between the length of time CO₂ was applied and the amount of time each spider remained anesthetized can be seen in Fig. 1. Curvefit analysis yielded the following power function:

$$\text{response (minutes)} = 0.36 \cdot \text{dosage (minutes)} - 0.98. \quad \text{eqn. (1)}$$

Both the constant (0.36) and the exponent (0.98) were significant ($P < 0.01$ and $P < 0.001$ respectively) and the R^2 -value for the curve was 0.74.

Because the curve approached linearity, a linear regression was used, yielding the following estimate of slope:

$$\text{response (minutes)} = 0.36 \cdot \text{dosage (minutes)} - 0.51. \quad (P < 0.001) \quad \text{eqn. (2)}$$

The upper and lower bounds of the 99% confidence interval for this slope (0.36) are 0.48 and 0.24, respectively. The (-0.51) value for the Y intercept did not differ significantly from the origin ($P > 0.05$). The R^2 -value for the linear relationship between dosage and response was 0.64.

The above results are useful for developing protocols requiring the spider to be safely incapacitated. From the slope, it can be estimated that spiders will remain anesthetized for 1 minute for every 3 minutes of CO₂ application. Because

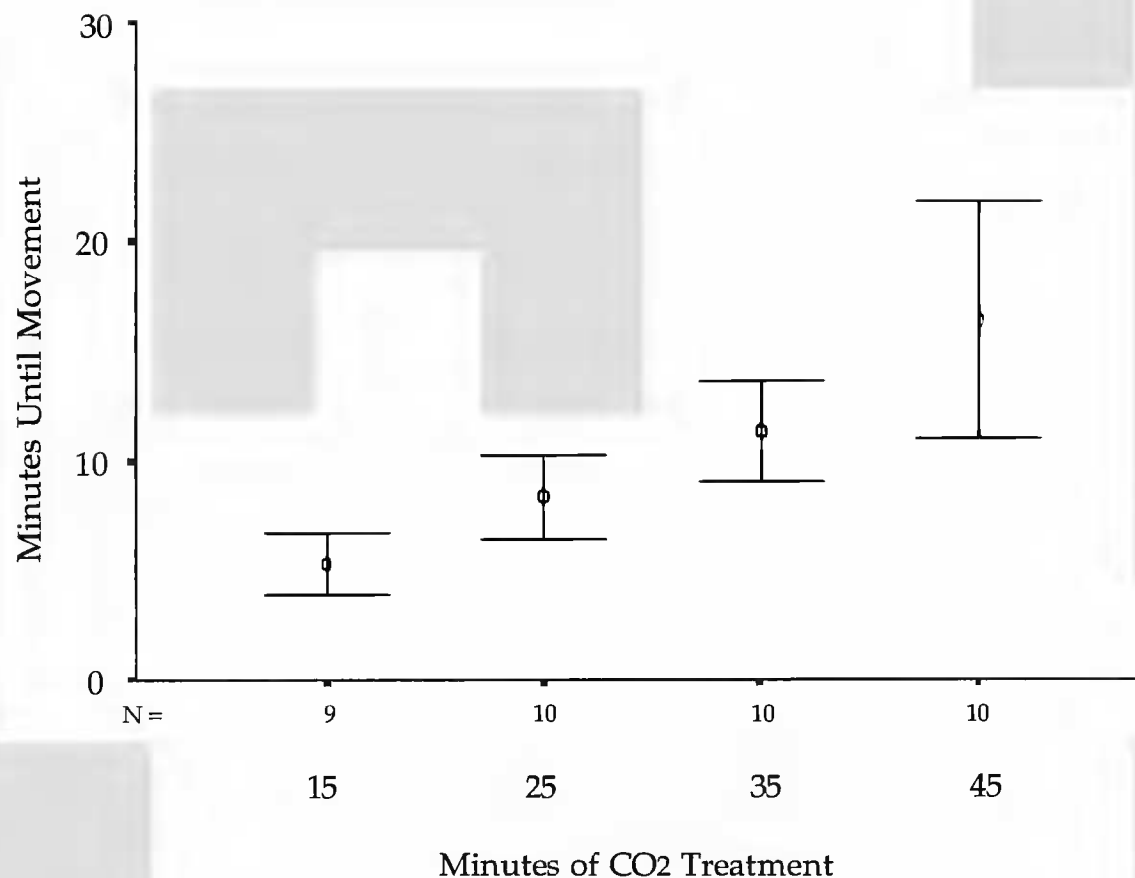


Figure 1. Relationship between dosage time (x-axis) and time until 1st movement. Error bars represent 99% confidence intervals.

of the amount of individual variation from the curve for waking times (low R^2 -values, see above), this curve should only be used as an estimate. For safety, the lower bound of the 99% confidence interval of equation (2), slope of 0.24, can be used. This yields expected anesthesia in a ratio of approximately 1 minute for every 4 minutes of CO₂ dosage.

Statistical tests were done to control for the effects of repeated use of the same specimens. Partial correlation between dosage and response controlling for specimen were only slightly different (R -value = 0.81) from the uncontrolled correlation (R -value = 0.80). Correlation between specimens and their dosage/response ratios across all four dosages approached but did not show a significant effect (P = 0.09).

The masses of the spiders in the range tested (130–990 mg) had no apparent effect on their dosage responses to CO₂. A correlation between dosage/response ratios and mass was insignificant (P = 0.32), and controlling for mass in a partial correlation between dosage/response ratios and mass yielded the same R -value (0.80) as in the uncontrolled test. Thus it appears to be unnecessary to find or estimate the mass of the spiders before determining the proper CO₂ dosage when using adult females with mass > 100 mg.

Based on metabolic data (Anderson 1970, 1994) from other theridiid spiders, including four other species of *Latrodectus*, it would not be prudent to assume this dosage/response relationship applies to other members of the genus. The metabolic rates (VO₂/h) can vary as much as 50% between *Latrodectus* species even when adjusted for allometric differences. Assuming that the spiders' response to CO₂ varies with CO₂ rates, the effects of this anesthetic treatment will vary in different species.

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